Chapter 10

Comparison of Volatile Analysis of Lipid-Containing and Meat Matrices by Solid Phase Micro- and Supercritical Fluid-Extraction

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Contamination and degradation of lipid moieties result in the formation of volatile compounds that affect the flavor and safety of food products. A wide variety of analytical techniques have been developed to determine the concentration of volatile flavor components in foods, such as vacuum distillation, headspace analysis, supercritical fluid extraction, and solid phase microextraction. Previously in our laboratory, volatile compounds from oxidized vegetable oil and fire/smoke damaged meat samples were analyzed by dynamic headspace analysis and supercritical fluid extraction (SFE). In this study, solid phase micro extraction (SPME) methods were also investigated to determine the concentration and identification of compounds from these samples. In applying SPME, different fiber types and analysis conditions were evaluated.

Solid phase microextraction (SPME) has recently been successfully utilized for analyzing many food substances and flavors (*1-4*) on a qualitative basis; however, quantitative studies are still limited. Recently Bartelt described quantitation of solutes by SPME and the difficulties that occur when doing quantitative determinations of headspace volatile for different classes of compounds (*5*). He found that the available fibers are not consistently responsive to all compounds, and equilibrium between the headspace and matrix for several compounds could not be attained at the conditions reported.

Various analytical methods for volatile components from lipids have been reported (*6-10*). Each of these methods have complexities, such as thermal degradation and/or instability of the components formed, that should be considered in developing the analysis method. In previous studies, we have shown that supercritical fluid extraction (SFE) of the volatile compounds has provided a means to quantitatively determine the concentration of

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lipid oxidation products (*8*). In a similar study, we have utilized SFE to determine aromatic hydrocarbons and polycyclic aromatic hydrocarbons (PAH) as marker compounds formed from the exposure of meats to smoke or fire conditions (*10*). However, the application of SPME to volatile compounds formed from lipid-containing samples has not been fully investigated (*11*). In this study, we have analyzed both a series of oxidized oils and meat samples by a SPME method followed by gas chromatoghraphy/mass spectrometry (GC/MS), and we have determined the effectiveness of this method compared to SFE and traditional purge and trap analysis.

Experimental methods

Samples. Canola oil, corn oil, soybean oil and sunflower oil were stored at 60°C in a forced draft oven until the peroxide values for canola, corn, and soybean were approximately 54, a value consistent with appreciable accelerated oxidation of the seed oils. The final peroxide value for sunflower oil under similar conditions was found to be 80 (*8*). Samples of each oil were also removed when the peroxide values were 2 and 18. Meat samples were obtained from the Food Safety and Inspection Service Laboratory in St. Louis, MO and were kept in a freezer at -45 °C. The samples included a smoked chicken product, ham, and corned beef, which were suspected of being exposed to a fire in an underground storage cavern (*10*). 50 Gram portions of the meat were removed from the original 300 g samples to obtain representative samples; the meat was ground and immediately frozen at -45 °C until analysis.

Standard solutions. Solutions of standard compounds including hydrocarbons and aldehydes, were prepared in concentrations from 1 ppb to 400 ppm to develop response curves for the major volatile compounds resulting from the oxidized oils. Also, solutions of 1 ppb to 400 ppb were prepared for aromatic hydrocarbons to determine the concentration of expected contaminants in the fire-exposed meats. All calibration solutions were formulated in a highly-stable hydrogenated soybean oil with a low volatile profile. The R^2 values of the calibration curves were 0.99 indicating a high degree of linearity (*12*). Dodecane at 1 ppm was added to each sample before analysis as an internal standard.

SPME analysis. In this study, three coated SPME fibers were evaluated: a 100 μm polydimethylsiloxane (PDMS), a 7 μm polydimethylsiloxane, and a 85 μm polyacrylate fiber (Supelco, Inc.; Bellefonte, PA). One-half gram samples, with 1ppm dodecane added as the internal standard, were placed into clear 10 mL vials from Supelco having teflon/silicone septa. Extraction conditions were varied to determine the optimal experimental parameters. Solutions of pentane, hexanal, nonanal, naphthalene and dodecane were preheated to 60 °C from 5 to 30 min using 5 min increments to provide different headspace concentrations. The preheating times were then plotted against peak area from the mass spectral data to assess the time required to reach equilibrium in the vial headspace. For example, as shown for the data for nonanal and dodecane plotted in Figure 1, the preheating time necessary for thermal equilibration was approximately 20 min. Similarly, pentane and hexanal reached equilibrium within 5 min, while naphthalene reached thermal equilibrium in 20 min. The time the SPME fiber was exposed to the headspace of

each standard varied from 5 min to 45 min to establish the best extraction time. Care was taken to determine that the equilibration time was sufficient for all analytes studied (*12*). The data from naphthalene and dodecane was also plotted against area from mass spectral data (Figure 2), and 30 min was determined to be the optimal extraction time. The optimum extraction time for the aldehydes used in this study was 20 min; however, a 30 min time was used for all samples.

Gas chromatography/mass spectrometry. SPME injections into the GC/MS system were made using a Varian 8200 Autosampler (Walnut Creek, CA). After the 30 min extraction time, the volatile compounds were desorbed for 1 min into the injector of a Varian Model 3600 GC equipped with a DB-5 capillary column (30 m, 0.25mm i.d., 0.25 μ film thickness) (J&W Scientific, Folsom, CA). The temperature of the column was maintained at 40 °C for 1 min during desorption then ramped at 5 °C/min to 220 °C. The injections were splitless with the injector temperature being held at 220 °C. The GC was interfaced with a Varian Saturn 4D Ion Trap MS/MS (Walnut Creek, CA) for detection and quantitation of the solutes. Mass spectral data were compiled using the electron impact mode.

Results and Discussion

The 100 μm PDMS coated fiber has been previously demonstrated to be a suitable fiber for detecting volatile compounds (*5, 13*). The 100 μm PDMS, the 85 μm polyacrylate and the 7 μm PDMS fibers were all used on a mixture of nine target compounds the first seven at a concentration of 10 ppm and dodecane at 1 ppm and hexadecane at 0.5 ppm concentrations (Figure 3). It is apparent from inspecting Figure 3 that the best overall response to the standard mixture is provided by using the 100μm PDMS fiber.

Bartelt has determined the calibration factors (K) for 71 analytes and determined that K was considerably greater for the higher molecular weight hydrocarbons than for aldehydes (*5*). Consequently, the area data from the mass spectral data for dodecane (1ppm) and hexadecane (0.5 ppm) are in much greater proportion to the other compounds measured at 10 ppm concentration levels, except for the compound 2-pentylfuran. The areas for all compounds, except for pentanal, were largest using the 100 μm PDMS fibers; the area for pentanal, a traditional indicator of oil oxidation, was highest with the polyacrylate fiber. The areas of the compounds with the 7 μm PDMS fiber were the smallest except for dodecane and hexadecane which tend to absorb preferentially on the non-polar PDMS fiber. The polyacrylate fiber tends to absorb the more polar analytes (*13*) and was found not to be as effective for the samples that we were studying (Figure 3).

The concentrations of several volatile components from four oxidized vegetable oils were measured using the 100 μm PDMS-coated fiber (Table 1). The concentration of volatiles increased for all compounds as the peroxide values increased during storage. Sunflower oil with 70% linoleic acid oxidizes the most rapidly; and the concentrations of hexanal and decadienal, oxidation products formed from linoleic acid, are greatest for sunflower oil. Nonanal was most prominent during the accelerated storage of canola oil. This is due to the fact that canola oil contains more than 60% oleic acid, the precursor for nonanal formation.

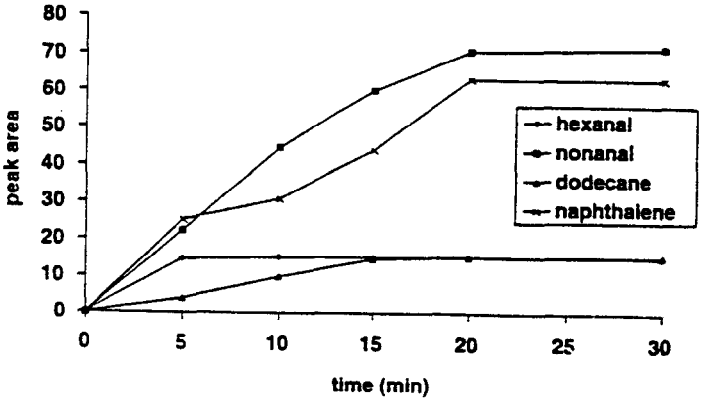

Figure 1. Effect of sample preheat time on equilibration of volatiles.

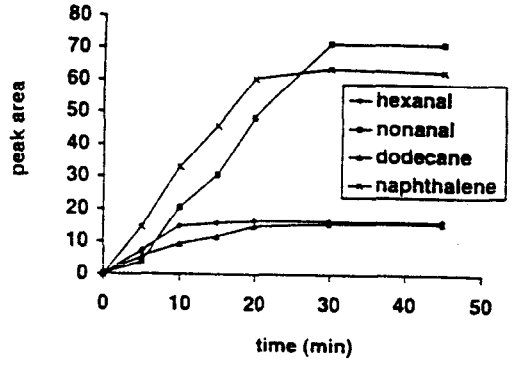

Figure 2. Effect of absorption time on equilibration of solute.

Table I. Volatile Concentration (ppm) in Oxidized Vegetable Oils by SPME Analysis

	Corn oil			Canola oil		
	PV=2	PV=19	PV=52	PV=2	PV=19	PV=58
pentane	0.09	0.44	0.96	0.35	0.65	0.67
pentanal	0.08	0.37	0.70	0.81	1.48	4.54
2-pentenal	0.20	0.30	0.54	0.31	0.27	0.28
hexanal	0.27	2.10	45.17	0.43	7.39	56.74
2-heptenal	0.19	0.48	1.28	0.27	0.37	0.47
2-pentylfuran	0.35	0.31	0.16	0.81	0.34	0.22
octanal	0.36	0.47	8.74	0.79	0.44	0.29
nonanal	1.20	7.13	12.65	0.10	0.91	2.17
2,4-decadienal	0.26	1.03	16.83	0.63	0.71	18.59

	Soybean oil			Sunflower oil			
	PV=2	PV=18	PV=54	PV=3	PV=18	PV=55	PV=81
pentane	0.06	0.44	1.03	0.23	0.34	3.23	5.36
pentanal	0.05	0.98	1.36	0.65	0.82	1.35	1.65
2-pentenal	0.08	0.16	0.37	0.11	0.21	0.32	0.48
hexanal	1.58	7.07	80.59	0.38	8.28	89.41	296.39
2-heptenal	0.96	1.49	15.50	0.14	0.27	2.17	5.49
2-pentylfuran	0.13	0.50	0.14	0.27	1.37	0.46	1.27
octanal	0.06	0.33	0.46	0.24	0.41	0.46	0.97
nonanal	0.73	1.28	2.48	0.09	0.87	1.16	2.01
2,4-decadienal	0.16	5.98	28.17	0.13	8.53	36.90	49.69

PV = peroxide value (meq/kg) a measure of oxidation in oils (8)

Values obtained for the compounds from oils that were highly oxidized and analyzed by an SFE method are shown in Table 2 (*8*). The data from SPME analysis tends to follow some of the same trends inherent in the oxidized oil data determined by SFE (*8*). The concentration of hexanal from the sunflower oil with a peroxide value of 80 was much lower when determined by SPME (296.39 ppm, Table I) than when determined by SFE (365.92ppm, Table II). However, 2-pentyl furan in all oils is up-to-10 fold higher in concentration as determined by SPME analysis relative to the SFE data.

Table II. Volatile Concentration of Oxidized Vegetable Oils by SFE Analysis (8)

	Canola oil	Corn oil	Soybean oil	Sunflower oil
	PV = 53	PV = 53	PV = 60	PV = 82
pentane	0.67	0.22	0.31	0.88
pentanal	2.12	0.90	0.89	1.23
hexanal	52.63	69.93	81.36	365.92
2-heptenal	1.32	2.89	6.97	10.90
2-pentyfuran	0.09	0.08	0.03	0.10
octanal	20.36	1.42	1.32	1.87
nonanal	26.98	0.94	4.51	5.42
2,4-decadienal	16.98	22.80	27.03	30.54

PV = peroxide value (meq/kg) a measure of oxidation in oils

Figure 4 compares the SPME results with those from SFE for the major vegetable oil volatiles produced from the four highly-oxidized oils used in this study. Overall these results show that the pattern of oxidation products formed and detected are very consistent using either SPME and SFE for extraction. Although there are subtle differences between the results from the two techniques, it appears in most cases that the two techniques agree within an order of magnitude for the major volatiles detected. These results indicate, that either SPME or SFE can be used with confidence to monitor the degradation products produced upon aging the oil matrices.

Zhang and Pawliszyn (*14*) demonstrated the SPME technique is highly sensitive to polycyclic aromatic hydrocarbons (PAH) found in environmental samples. Previously we have examined meat samples that were exposed to fire or smoke by an SFE method and found ppb levels of these compounds (*10*). Therefore, SPME was applied to analyze specifically for aromatic compounds in both fire-exposed and control meat samples.

Three of the previously analyzed meat products and their analysis by SPME are listed in Table III. The SPME method proved effective in detecting the aromatic hydrocarbons found previously via SFE in the three meat matrices. The values for both the aromatic hydrocarbons and naphthalene by SPME tend to follow the same trend as that reported by SFE (Table IV) (*10*), with the exception of the values of naphthalene for corned beef. The SPME technique was able to measure lower concentrations than found by the SFE method, especially in the control samples. Also, methylnaphthalene, previously reported by purge and trap headspace analysis to be present in the fire exposed samples (*15*), was not found by the SFE method (*10*). However, using SPME, 1-methylnaphthalene was identified and its presence determined as low as 1 ppb in the corned beef sample.

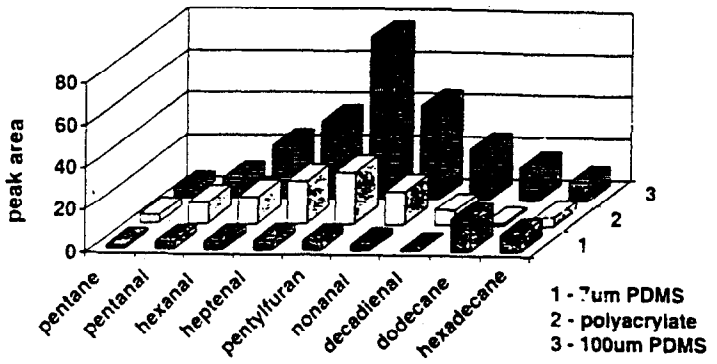

Figure 3. Effect of fiber type on solute extraction.

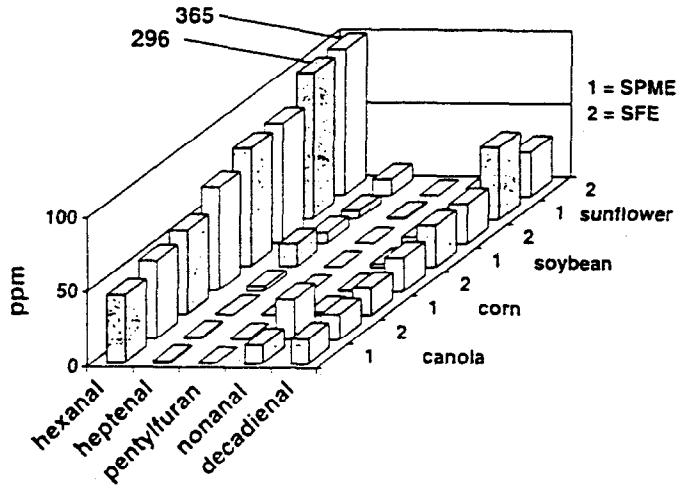

Figure 4. SPME vs SFE analysis of oxidized oil volatiles.

Table III. Aromatic Hydrocarbons (ppb) in Meats Exposed to Fire/Smoke by SPME

	Smoked Chicken		Ham		Corned Beef	
	control	fire*	control	fire*	control	fire*
benzene	7	51	8	44	1	3
toluene	30	98	1	46	38	49
xylene	4	100	4	13	3	7
ethylbenzene	5	14	4	16	4	12
butylbenzene	15	41	22	33	2	5
naphthalene	14	50	3	13	3	2
1-methylnaphthalene	12	23	20	11	2	1

* fire = samples exposed to fire/smoke.

Table IV. Aromatic Hydrocarbons (ppb) in Meats Exposed to Fire/Smoke by SFE

	Smoked Chicken		Ham		Corned Beef	
	control	fire*	control	fire*	control	fire*
benzene	2	43	8	37	2	4
toluene	29	329	1	80	22	52
xylene	26	164	6	15	1	19
ethylbenzene	11	250	2	41	4	31
naphthalene	10	39	5	21	0	7
1-methylnaphthalene	N.D.[1]					

* fire = samples exposed to fire/smoke.
[1] N.D. = not detected

In summary, SPME using the 100 μm PDMS fiber has been shown to efficiently extract and measure volatile compounds in lipid-containing matrices at levels equivalent to those found by our previously-described SFE method. The SPME technique, as with the SFE method, uses moderate extraction temperatures that do not degrade lipid moieties or produce artifacts due to the analytical technique. Therefore, this method can be used in place of the traditional purge and trap method that uses higher temperatures or longer collection times for the extraction and determination of volatile compounds in lipids. In addition, both the SPME and SFE techniques are environmentally benign, use minimal quantities of solvent, and can complement one another for true analysis of analytes (*16*).

However, sample preparation time is shorter with SPME thus SPME is a simpler process than SFE; therefore more samples can be analyzed by SPME than with SFE.

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